P153

Site-directed Mutagenesis of Cofactor-binding Motif in Triphenylmethane Reductase (TMR)

Moon-Sun Jang[2], Nam-Young Kang[1], Kyoung-Sook Kim[1] and Young-Choon Lee[1,2]

[1]Brain Korea 21 Center for Silver-Bio Industrialization and [2]Department of Biotechnology, Dong-A University, Busan 604-714, South Korea

Triphenylmethane reductase (TMR) catalyzes the NADH-dependent reduction of triphenylmethane dyes. Sequence alignment revealed a region with a conserved GXXGXXG motif near its N-terminus, which corresponds to a conserved structural motif of known dinucleotide-binding proteins. To verify whether some of these glycine residues are important for the enzyme catalysis, these three glycine residues (Gly-7, Gly-10 and Gly-13) were individually replaced by alanine using site-directed mutagenesis. The secondary structures of these mutants as measured by circular dichroism (CD) spectroscopy did not show remarkable differences as compared with the wild type. The Vmax/Km values of mutants G7A and G13A for both Basic fuchsin and NADH were increased about 3- and 2-fold over that of the wild type, respectively, whereas the Vmax/Km value of mutant G10A were decreased about 6-fold. These results suggest that these three glycine residues are involved in the interaction with both substrate and cofactor for the catalytic activity of TMR.

Key words: Triphenylmethane reductase, cofactor, site-directed mutagenesis

P154

Effects of Physico-chemical Parameters on the Color Removal of Real Dye Effluent by *Citrobacter* sp. Strain KCTC 18061P

Moon-Sun Jang[2], Nam-Young Kang[1], Kyoung-Sook Kim[1], Byung-Gil Jung[3], Nak-Chang Sung[3] and Young-Choon Lee[1,2]

[1]Brain Korea 21 Center for Silver-Bio Industrialization and
[2]Department of Biotechnology, Dong-A University, Busan 604-714, South Korea,
[3]Department of Environmental Engineering, College of Engineering, Dong-A University, Busan 604-714, South Korea

Effects of physico-chemical parameters, such as aeration, nitrogen source, glucose and effluent concentrations on the color removal of real dye effluent by this strain were investigated. The observed changes in the visible spectra indicated color removal by the absorption of dye to cells during incubation with the strain. This strain showed higher decolorization ability under aerobic than static culture conditions. With 1% glucose, this strain removed 70% of effluent color within 5 days. Decolorization was not significantly dependent on the nitrogen sources tested. Chemical oxygen demand (COD) and biological oxygen demand (BOD) were reduced in proportion to incubation times, and their reduction rates were about 35% and 50%, respectively, at 7 days of culture.

Key words: *Citrobacter* sp. strain KCTC 18061P, decolorization, real dye effluent